Analysis of Genetic Variation in Populations of Olympic Mudminnow

Patrick DeHaan, Brice Adams, Denise Hawkins
U.S. Fish and Wildlife Service
Abernathy Fish Technology Center
Longview, WA

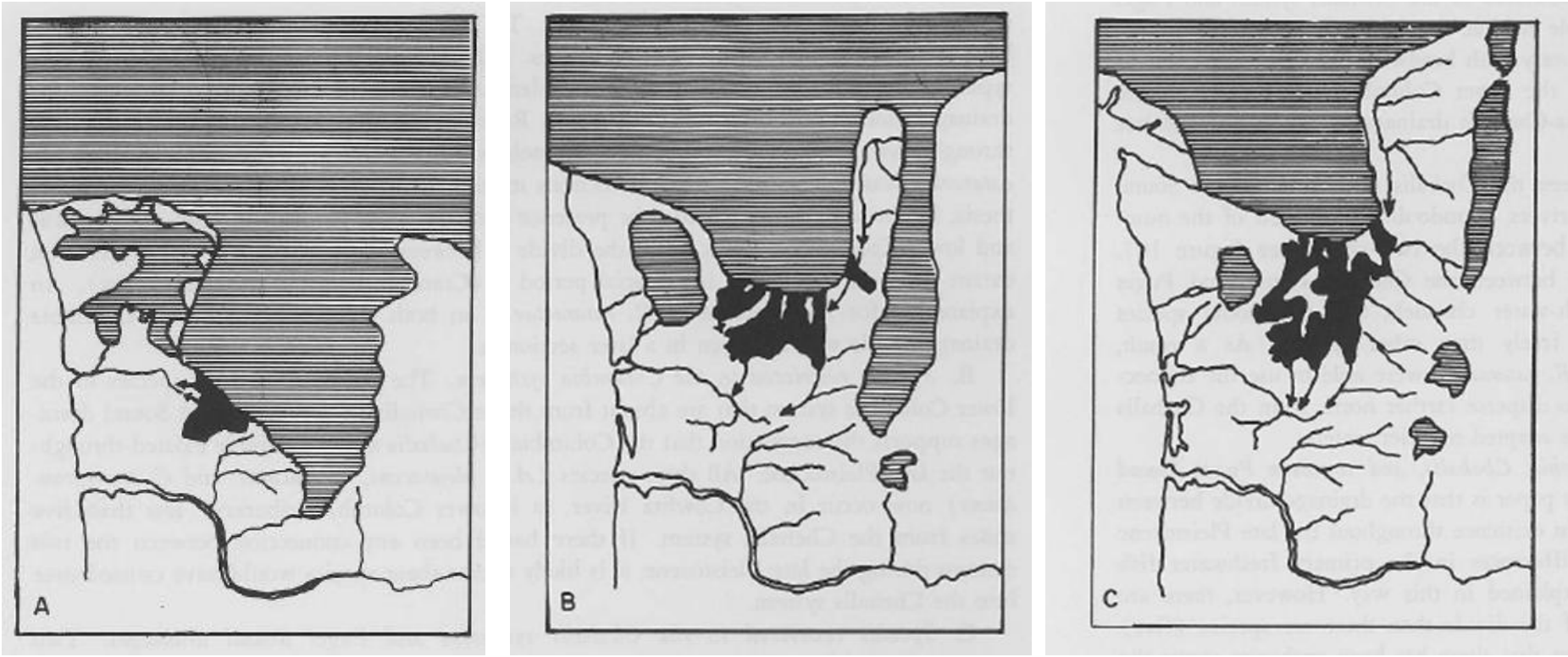
Roger Tabor, Brad Thompson
U.S. Fish and Wildlife Service
Washington Fish and Wildlife Office
Lacey, WA

Photo credit: Roger Tabor

Objectives

- Describe the levels of genetic diversity within mudminnow collections from throughout the species range
- Determine the spatial scale that constitutes a "population" of Olympic mudminnow
- Determine the major genetic groups for Olympic mudminnow to aid with possible designation of conservation units
- Determine the origins of Olympic mudminnow populations located on the Eastern side of Puget Sound

How Did Glacial History Affect Olympic Mudminnow?

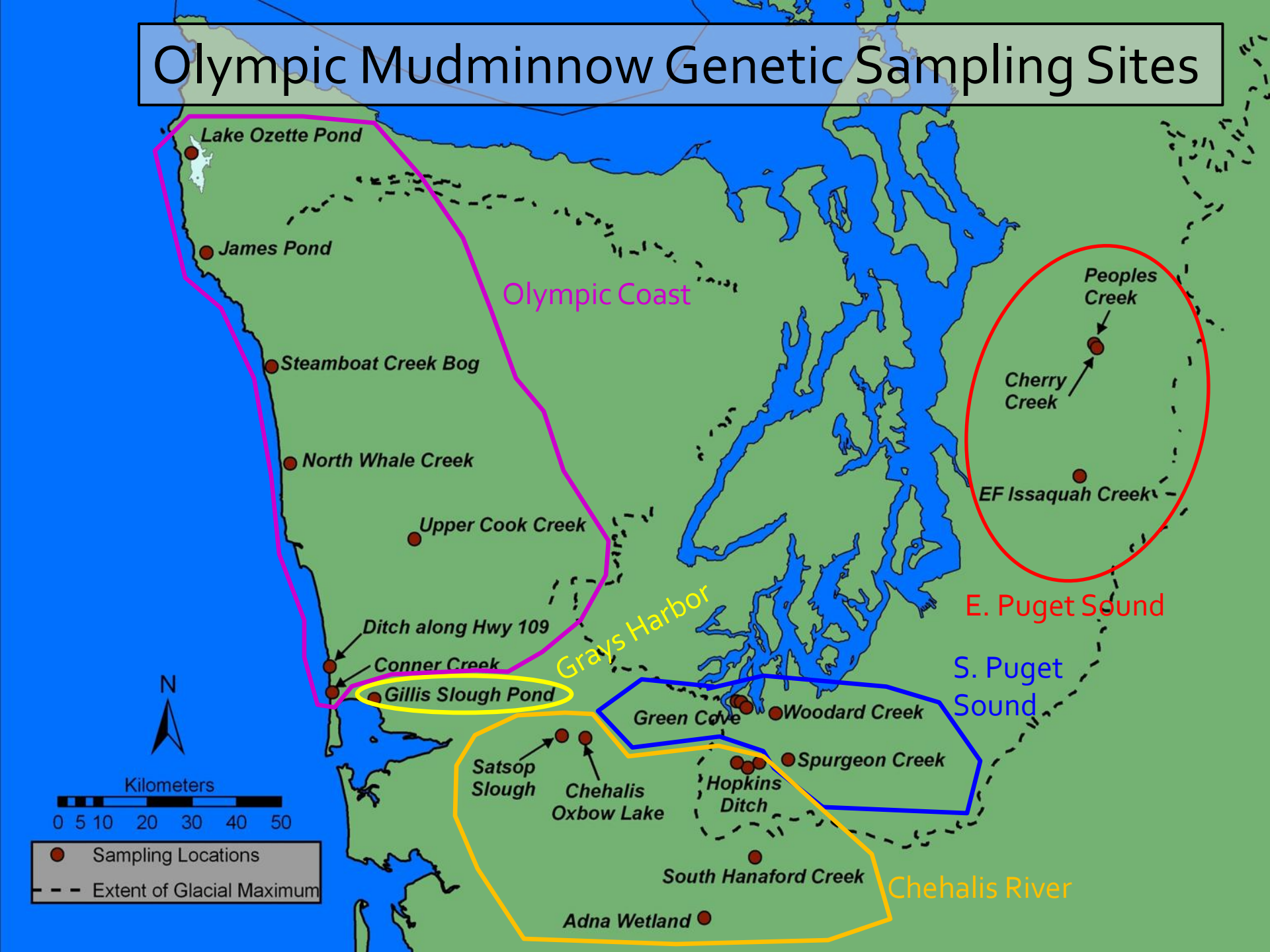


From McPhail (1967) NW Sci 41:1-11

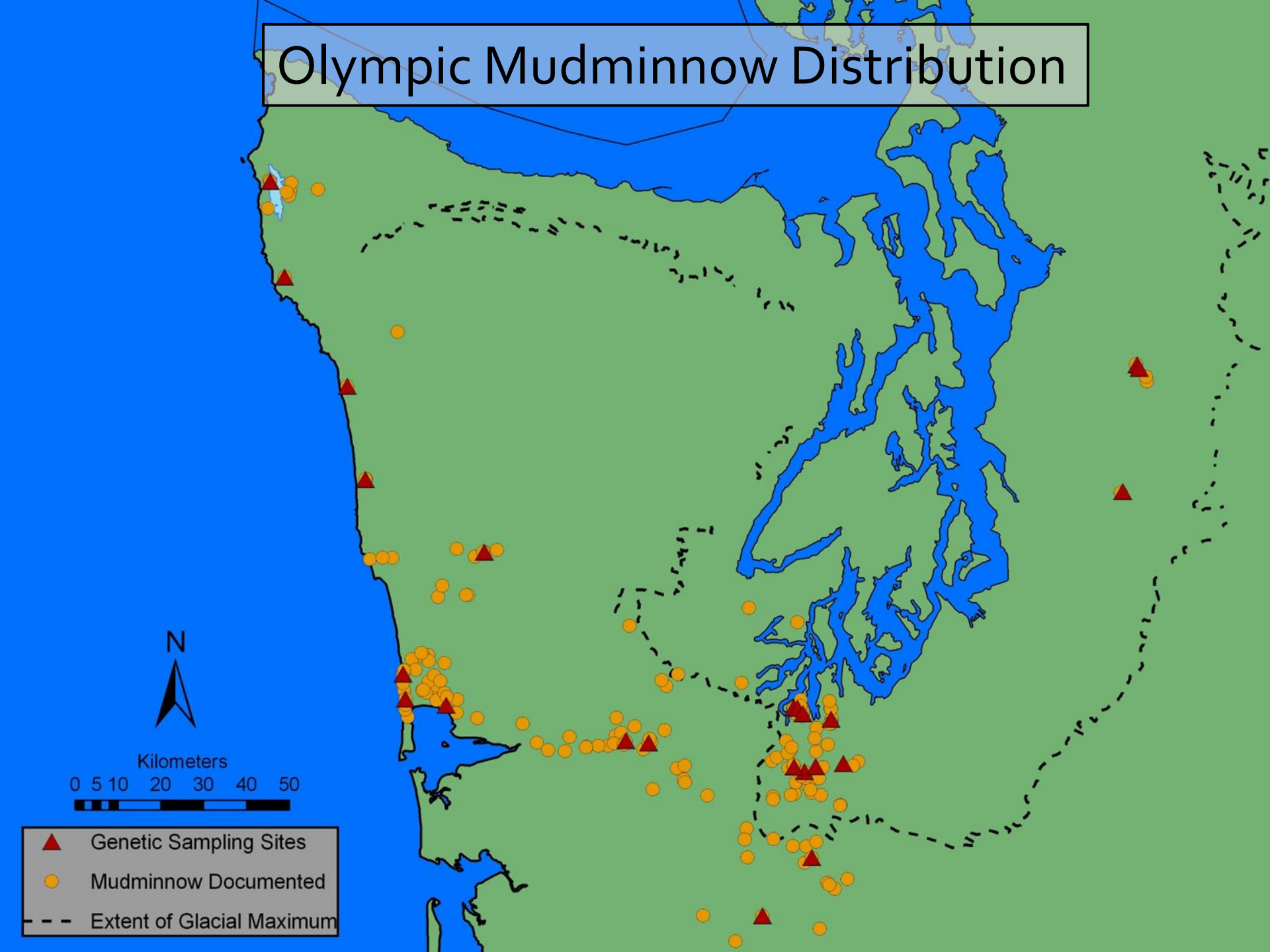
Methods

- Olympic mudminnow collected at 23 sites throughout the species range
  - Chehalis Basin (n = 7)
  - South Puget Sound (n = 5)
  - East Puget Sound (n = 3)
  - Grays Harbor (n = 1)
  - Olympic Coast (n = 7)
- Targeted 50 individuals per site
- Minnow traps, dip nets, and electrofishing used
- Multiple collections made at Green Cove Wetland and Hopkins Ditch (1-3 km apart)
- Developed new microsatellite DNA markers specifically for Olympic mudminnow
  - 13 loci used for genetic analysis

Olympic Mudminnow Genetic Sampling Sites



Olympic Mudminnow Distribution



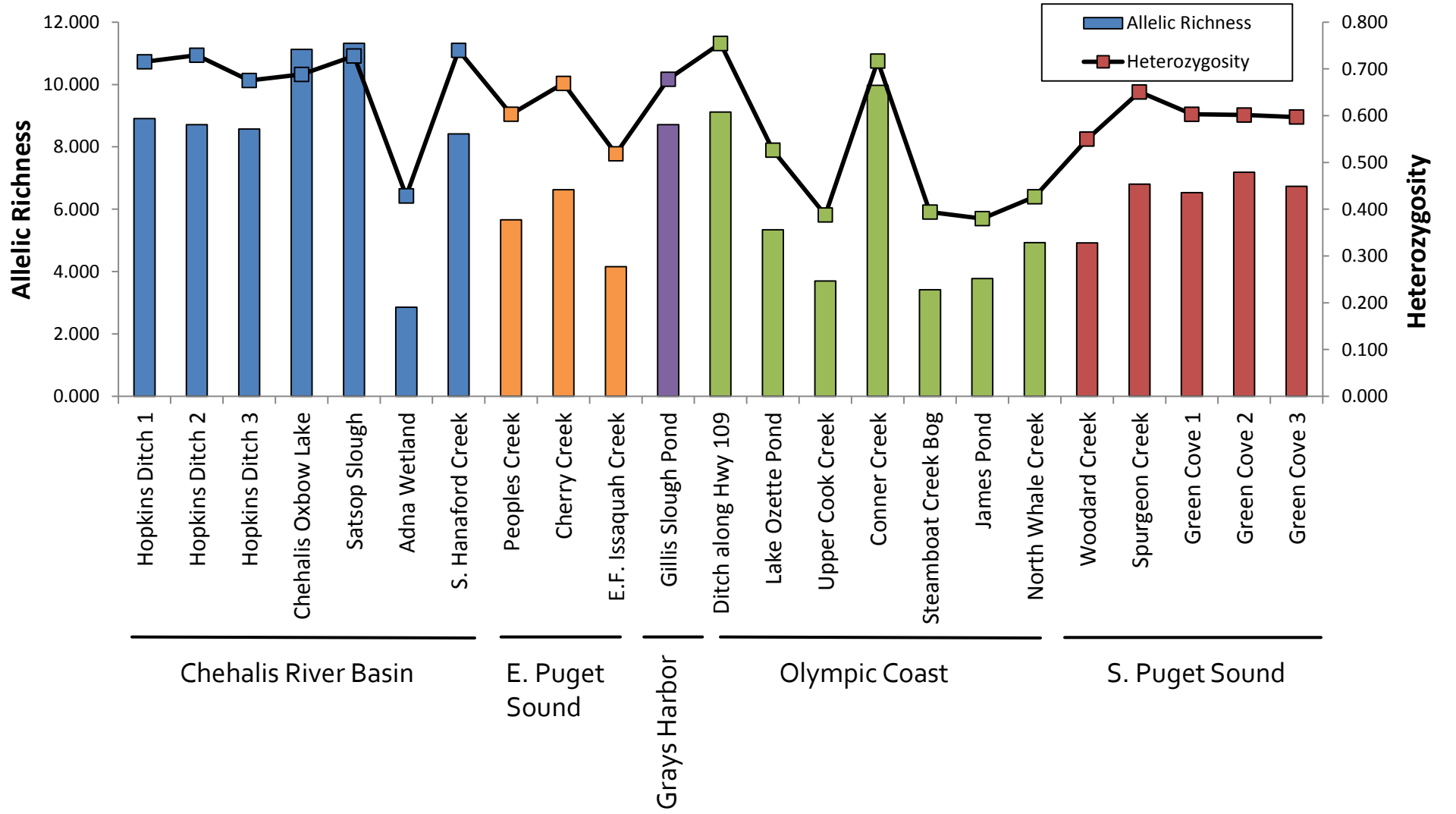
Objectives

- Describe the levels of genetic diversity within mudminnow collections from throughout the species range
- Determine the spatial scale that constitutes a "population" of Olympic mudminnow
- Determine the major genetic groups for Olympic mudminnow to aid with possible designation of conservation units
- Determine the origins of Olympic mudminnow populations located on the Eastern side of Puget Sound

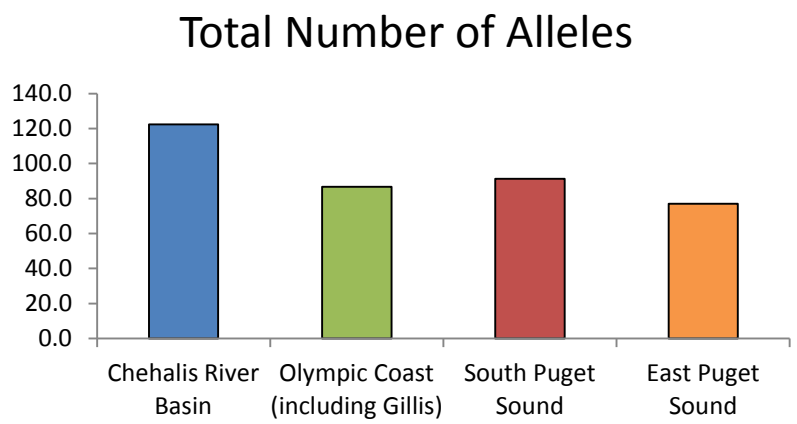
Why Monitor Genetic Diversity?

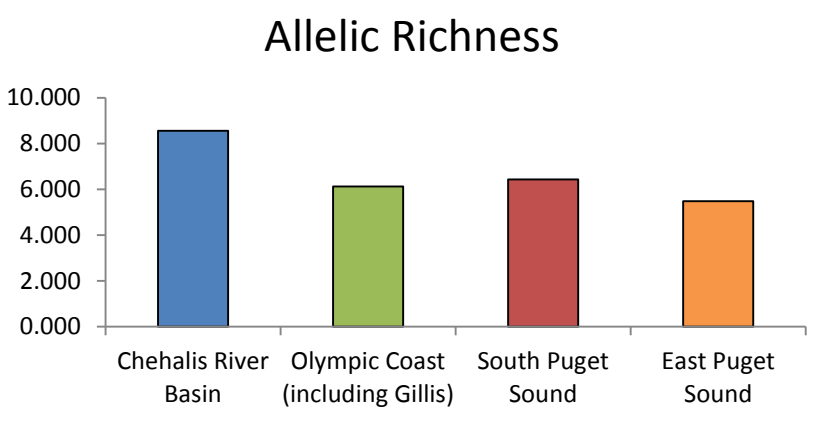
- Populations with increased genetic diversity may show increased fitness
- Populations with increased genetic diversity may be better suited for future environmental changes (e.g. climate change, habitat alteration)
- Detection of inbreeding and population bottlenecks
- Trends in genetic diversity can be monitored over time to assess population status

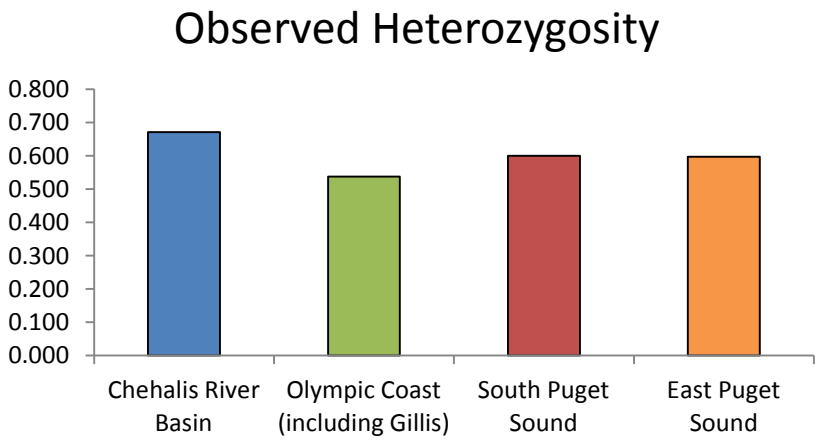
Genetic Diversity

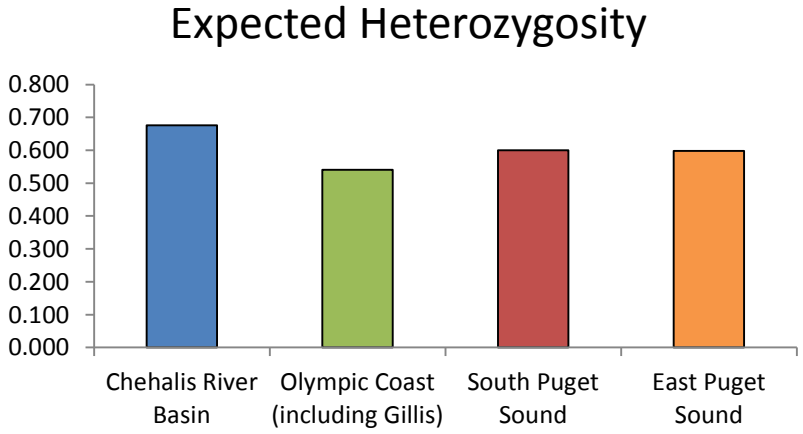


Comparisons Among Drainages









Genetic Bottleneck Tests

- All sites tested for evidence of a recent genetic bottleneck
  - Tests based on heterozygote excess
  - Able to detect a bottleneck within approximately the last 4 generations
- Two of 23 sample sites showed evidence of a recent genetic bottleneck
  - James Pond: dried up multiple times in recent years
  - S. Hanaford: fewer fish observed compared to other Chehalis Basin sites

Objectives

- Describe the levels of genetic diversity within mudminnow collections from throughout the species range
- Determine the spatial scale that constitutes a "population" of Olympic mudminnow
- Determine the major genetic groups for Olympic mudminnow to aid with possible designation of conservation units
- Determine the origins of Olympic mudminnow populations located on the Eastern side of Puget Sound

What Constitutes a Population?

- Mudminnow collected at multiple spatial scales
  - Broad geographic areas (Chehalis, S. Puget Sound, etc.)
  - Sites within a geographic area (Green Cove, Woodard, Spurgeon, etc.)
  - Multiple collections within Green Cove & Hopkins Ditch sites
- At what spatial scale do we observe significant genetic variation among collections?
- Is the level of variation among collections consistent across the species range?

What Constitutes a Population?

- Overall level of genetic variation among sample sites (F_{ST}) was 0.273 (95% C.I. = 0.200 - 0.361)

- The level of variation among sites varies by geographic area

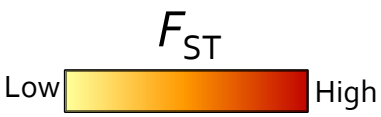
Geographic area	F_{ST}
Chehalis River	0.101
S. Puget Sound	0.167
E. Puget Sound	0.089
Olympic Coast (including Gillis Slough)	0.350

What Constitutes a Population?

- Pairwise estimates of F_{ST} ranged from 0.002 to 0.539
- Significant allele frequency differences among nearly all sampling locations
  - No significant difference among Green Cove sites
  - No Significant difference among Hopkins Ditch sites
- Nearly all sampling locations represent a genetically distinct population
  - Green Cove and Hopkins Ditch each represent a single population

Pairwise F_{ST} Comparisons

	Chehalis							E. Puget Sound			Grays Harbor	Olympic Coast							S. Puget Sound			
Chehalis	0.003																					
	0.004	0.002																				
	0.068	0.073	0.078																			
	0.074	0.071	0.075	0.055																		
	0.248	0.236	0.246	0.212	0.226																	
	0.037	0.023	0.033	0.070	0.063	0.235																
E. Puget Sound	0.268	0.257	0.264	0.230	0.243	0.347	0.242															
	0.225	0.215	0.221	0.180	0.189	0.308	0.210	0.061														
	0.319	0.315	0.316	0.277	0.287	0.412	0.307	0.126	0.080													
Grays Harbor	0.190	0.186	0.193	0.162	0.173	0.311	0.185	0.154	0.096	0.198												
Olympic Coast	0.168	0.167	0.173	0.150	0.160	0.295	0.169	0.179	0.139	0.226	0.135											
	0.342	0.332	0.343	0.330	0.328	0.461	0.330	0.378	0.350	0.420	0.320	0.314										
	0.339	0.337	0.332	0.317	0.322	0.472	0.347	0.301	0.252	0.325	0.235	0.277	0.472									
	0.167	0.166	0.170	0.145	0.145	0.292	0.159	0.200	0.149	0.244	0.138	0.040	0.341	0.279								
	0.372	0.362	0.371	0.370	0.354	0.516	0.351	0.372	0.366	0.445	0.360	0.343	0.393	0.498	0.358							
	0.390	0.378	0.386	0.365	0.353	0.487	0.394	0.433	0.409	0.485	0.384	0.398	0.373	0.539	0.415	0.529						
	0.369	0.361	0.366	0.345	0.336	0.482	0.377	0.414	0.389	0.465	0.364	0.372	0.349	0.515	0.391	0.500	0.030					
S. Puget Sound	0.207	0.186	0.202	0.228	0.216	0.315	0.167	0.290	0.282	0.385	0.263	0.242	0.382	0.402	0.222	0.335	0.472	0.454				
	0.088	0.078	0.092	0.131	0.102	0.279	0.067	0.302	0.276	0.369	0.230	0.220	0.362	0.378	0.208	0.394	0.414	0.396	0.210			
	0.095	0.094	0.100	0.136	0.134	0.286	0.110	0.301	0.262	0.357	0.272	0.222	0.401	0.401	0.222	0.441	0.440	0.421	0.251	0.170		
	0.102	0.107	0.107	0.146	0.142	0.302	0.123	0.303	0.264	0.354	0.281	0.229	0.410	0.397	0.229	0.445	0.441	0.421	0.261	0.182	0.006	
	0.103	0.108	0.108	0.146	0.145	0.296	0.124	0.300	0.262	0.351	0.279	0.228	0.408	0.398	0.230	0.443	0.443	0.424	0.268	0.187	0.002	0.003



Comparisons to Other Species

Species	Overall F_{ST}	Range Pairwise F_{ST} values	Source
Pink salmon	0.02	0.000-0.098	Olsen et al.
Chinook salmon	0.067		Narum et al. 2008
Green sturgeon		0.001-0.085	Israel et al. 2009
Oregon chub	0.078	0.000-0.250	DeHaan et al. 2012
Coastal cutthroat trout	0.121		Wenburg et al. 1998
Olympic mudminnow	**0.273**	**0.002-0.539**	**This study**
Bull trout	0.32	0.03-0.62	Ardren et al. 2011
Westslope cutthroat trout	0.32	0.00-0.78	Taylor et al. 2003

Estimates based on microsatellite studies covering broad geographic areas

Objectives

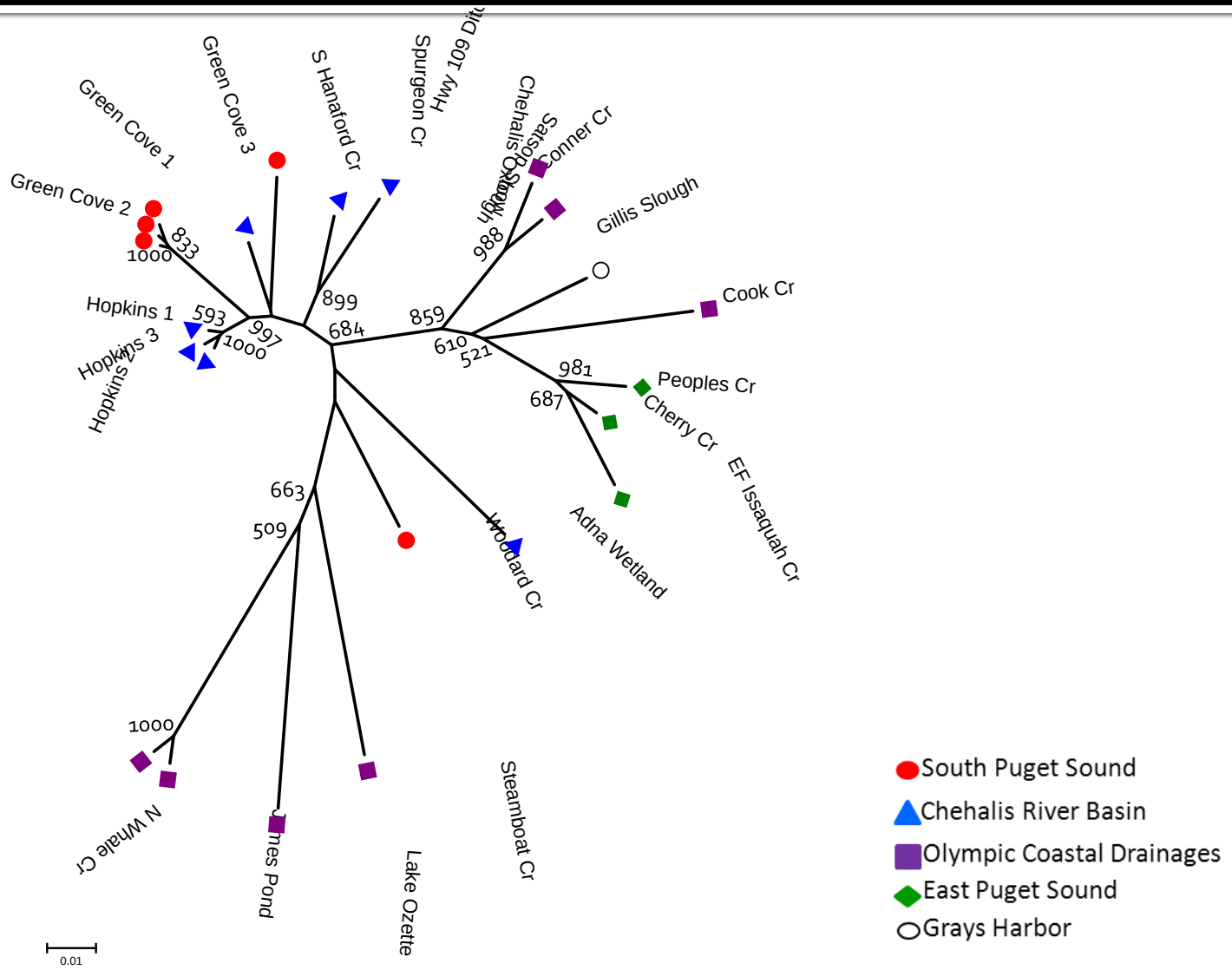
- Describe the levels of genetic diversity within mudminnow collections from throughout the species range
- Determine the spatial scale that constitutes a "population" of Olympic mudminnow
- Determine the major genetic groups for Olympic mudminnow to aid with possible designation of conservation units
- Determine the origins of Olympic mudminnow populations located on the Eastern side of Puget Sound

FCA


Roger Tabor

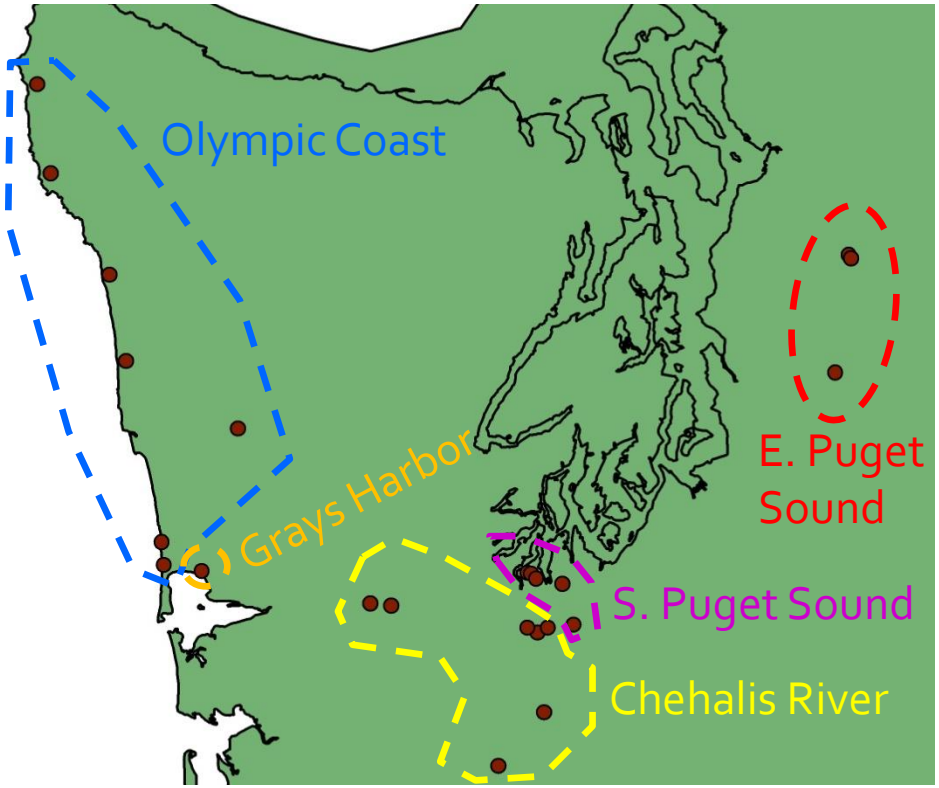
13.19%
17.93%
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-2
-1.5
-1
-0.5
0
0.5
1
Hopkins Ditch 1
Hopkins Ditch 2
Hopkins Ditch 3
Chehalis Oxbow Lake
Satsop Slough
Adna Wetland
S Hanaford Creek
Peoples Creek
Cherry Creek
EF Issaquah Creek
Gillis Slough
Hwy 109 Ditch
Lake Ozette Pond
Upper Cook Creek
Conner Creek
Steamboat Creek
James Pond
N Whale Creek
Woodard Creek
Spurgeon Creek
Green Cove 1
Green Cove 2
Green Cove 3

Neighbor-Joining Tree



AMOVA Analysis #1

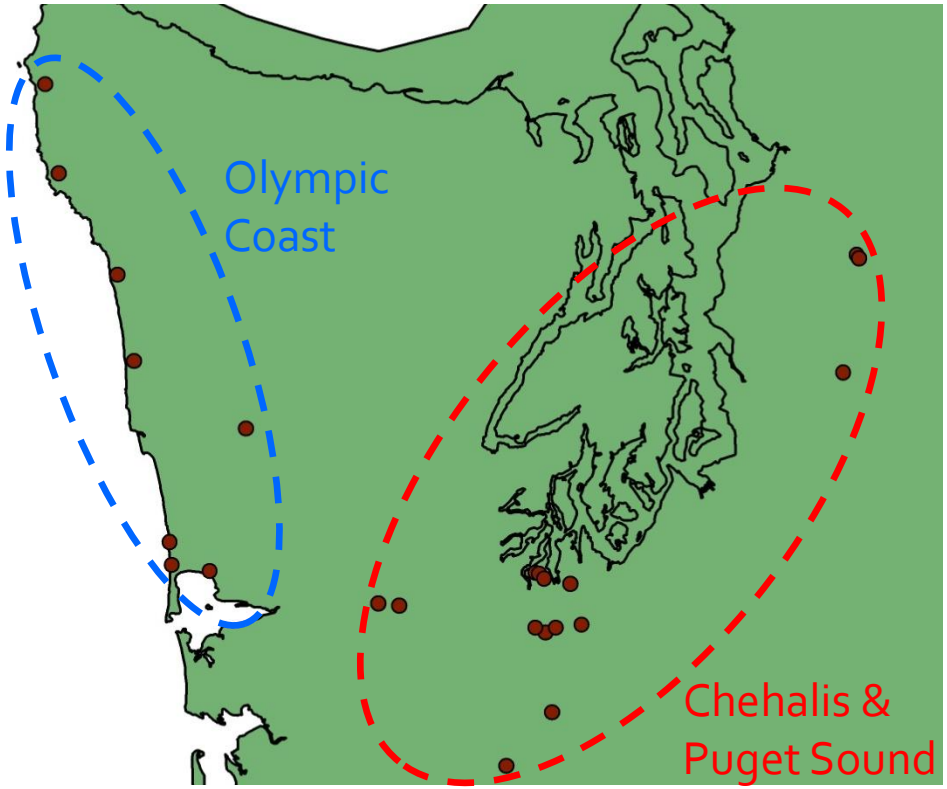
5 groups organized by geographic area



Source of Variation	Percentage of Variation	Fixation Indices
Among Groups	9.82%	F_{CT} = 0.098
Among Pops w/in Groups	19.00%	F_{SC} = 0.211
Among Individuals w/in Pops	0.21%	F_{IS} = 0.003

AMOVA Analysis #2

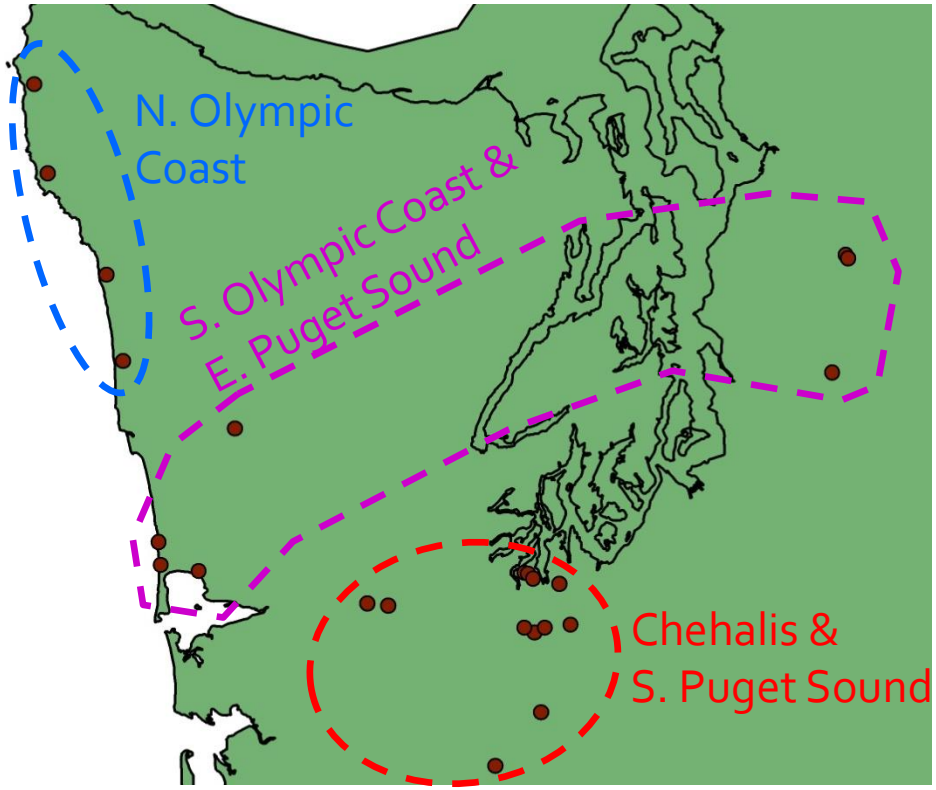
Coastal populations vs. Chehalis and Puget Sound populations



Source of Variation	Percentage of Variation	Fixation Indices
Among Groups	6.64%	F_{CT} = 0.066
Among Pops w/in Groups	23.21%	F_{SC} = 0.249
Among Individuals w/in Pops	0.20%	F_{IS} = 0.003

AMOVA Analysis #3

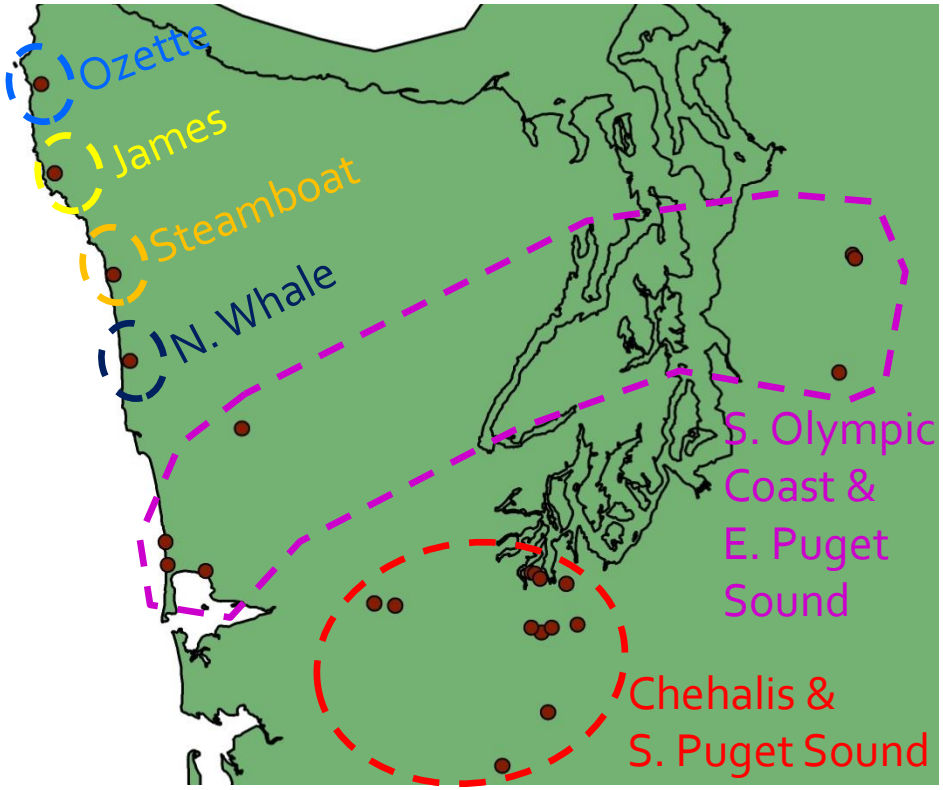
3 groups organized by FCA & NJ tree clusters



Source of Variation	Percentage of Variation	Fixation Indices
Among Groups	15.83%	F_{CT} = 0.158
Among Pops w/in Groups	15.73%	F_{SC} = 0.187
Among Individuals w/in Pops	0.20%	F_{IS} = 0.003

AMOVA Analysis #4

6 groups: FCA & NJ Tree clusters with N. Olympic populations as separate groups

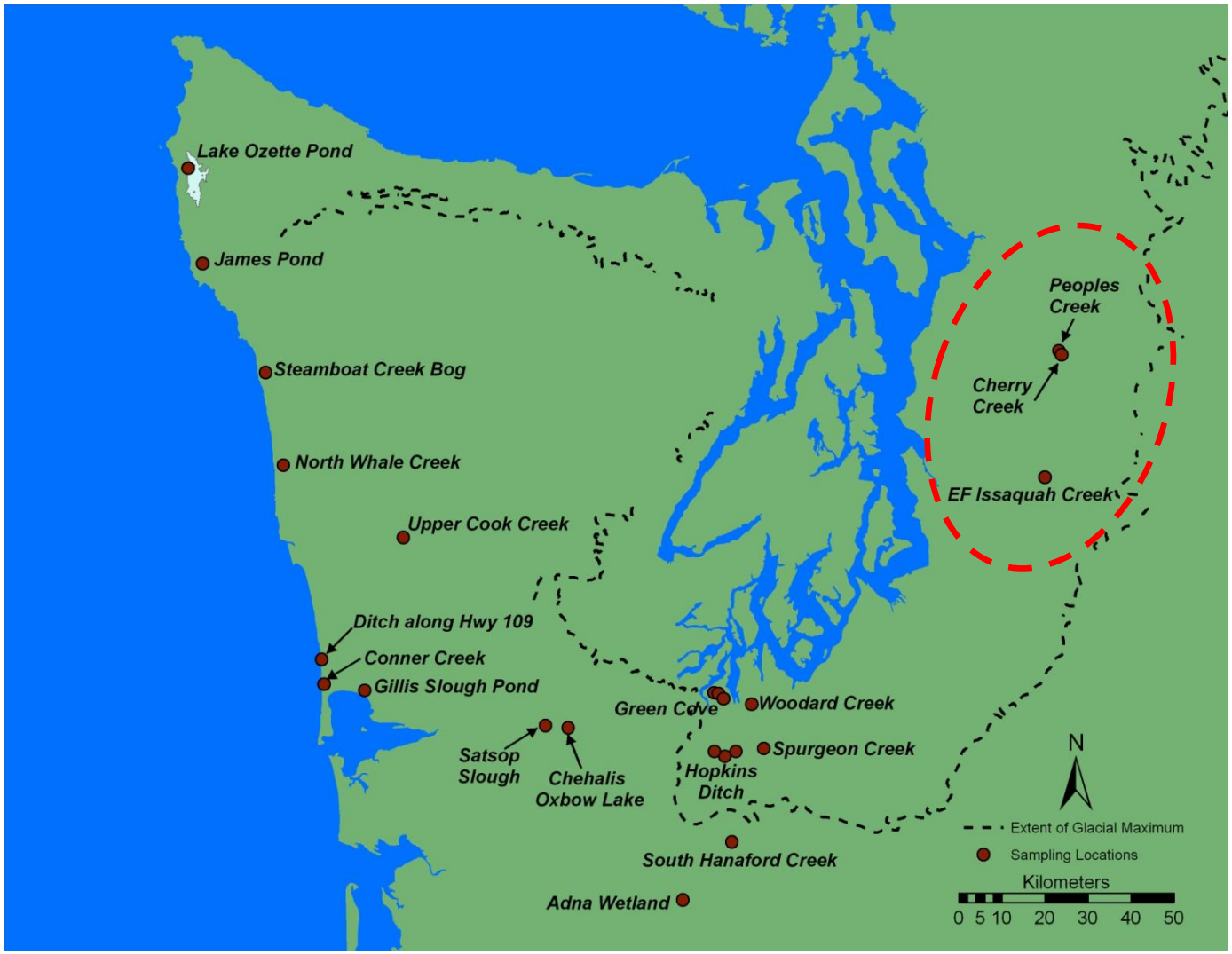


Source of Variation	Percentage of Variation	Fixation Indices
Among Groups	18.78%	F_{CT} = 0.188
Among Pops w/in Groups	13.28%	F_{SC} = 0.164
Among Individuals w/in Pops	0.20%	F_{IS} = 0.003

Objectives

- Describe the levels of genetic diversity within mudminnow collections from throughout the species range
- Determine the spatial scale that constitutes a "population" of Olympic mudminnow
- Determine the major genetic groups for Olympic mudminnow to aid with possible designation of conservation units
- Determine the origins of Olympic mudminnow populations located on the Eastern side of Puget Sound

Origins of E. Puget Sound Populations

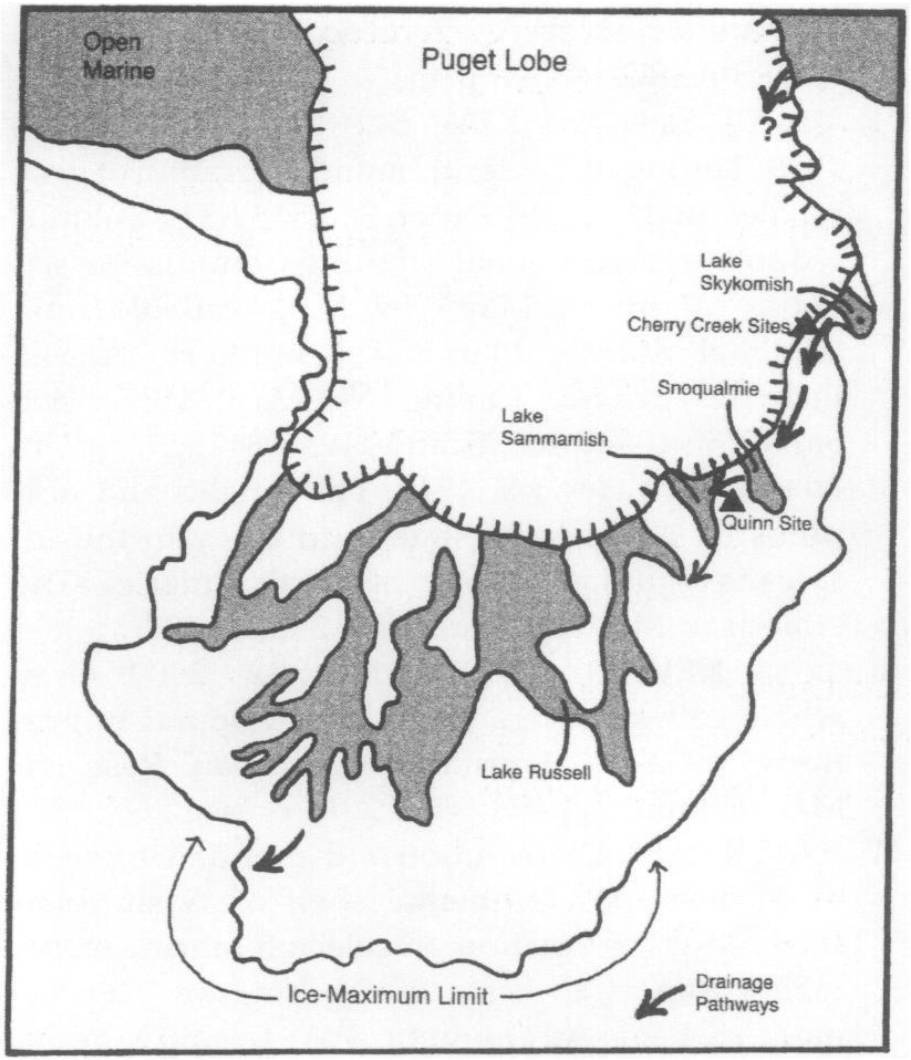


Origins of E. Puget Sound Populations

- WDFW 1999 Mudminnow Status Report (Mongillo and Hallock)
  - Mudminnow avoid current and would likely have avoided the swift flowing waters to access this habitat
  - No mudminnow found outside the known range in areas North of the Nisqually River
  - No mudminnow discovered during a rotenone treatment of Margaret Lake downstream of the Cherry Creek site
  - Mudmminnow only found at 1 site greater than 110m elevation; Cherry Cr. at 240m, E.F. Issaquah at 135m
- Until proven otherwise, assume these populations represent illegal introductions

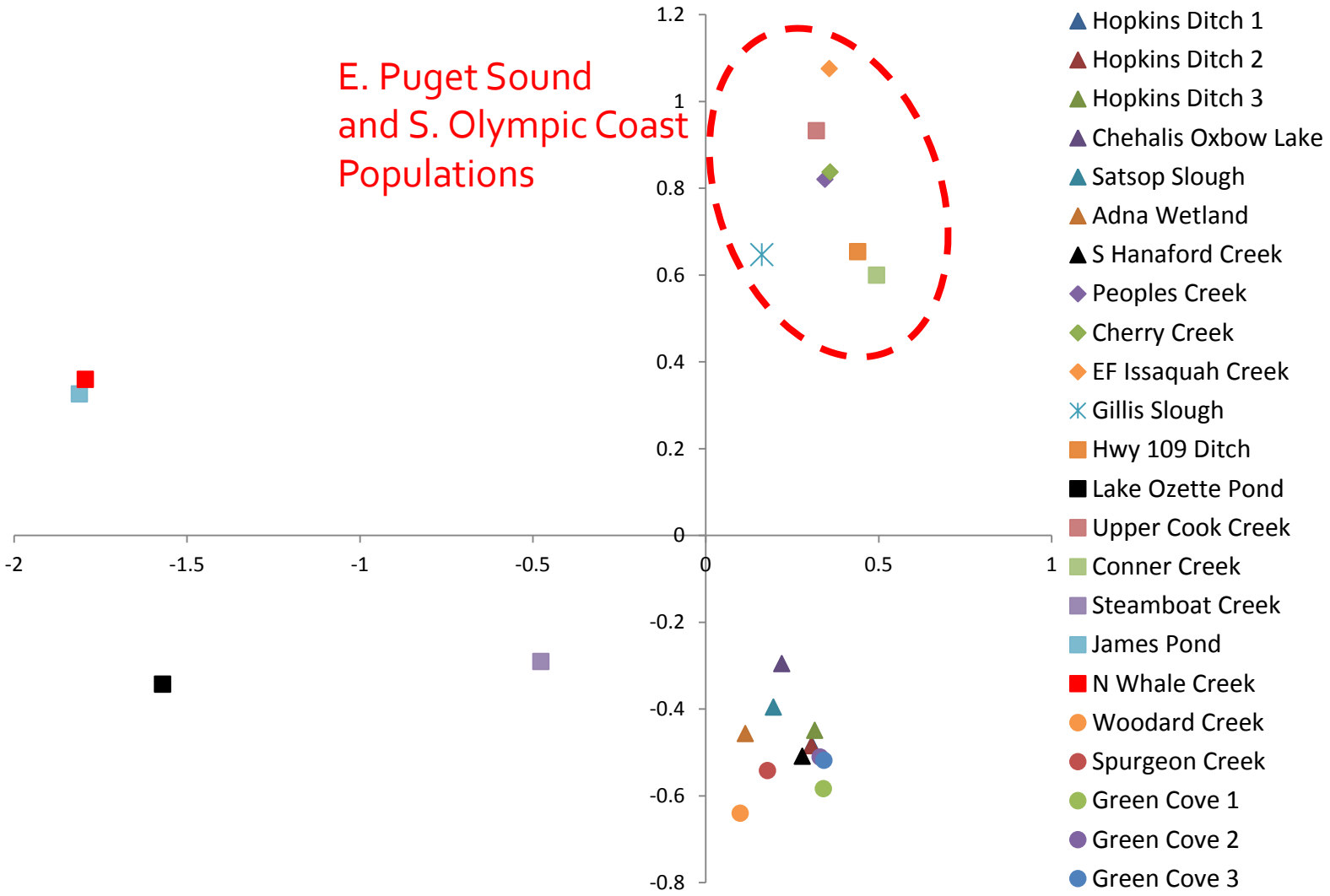
Origins of E. Puget Sound Populations

- Trotter et al. 2000
  - Mudminnow could have recolonized this area from Chehalis refugium via south flowing waterways
  - These sites could represent relict populations that persisted at glacial margins
- Genetic analysis could help to resolve this question

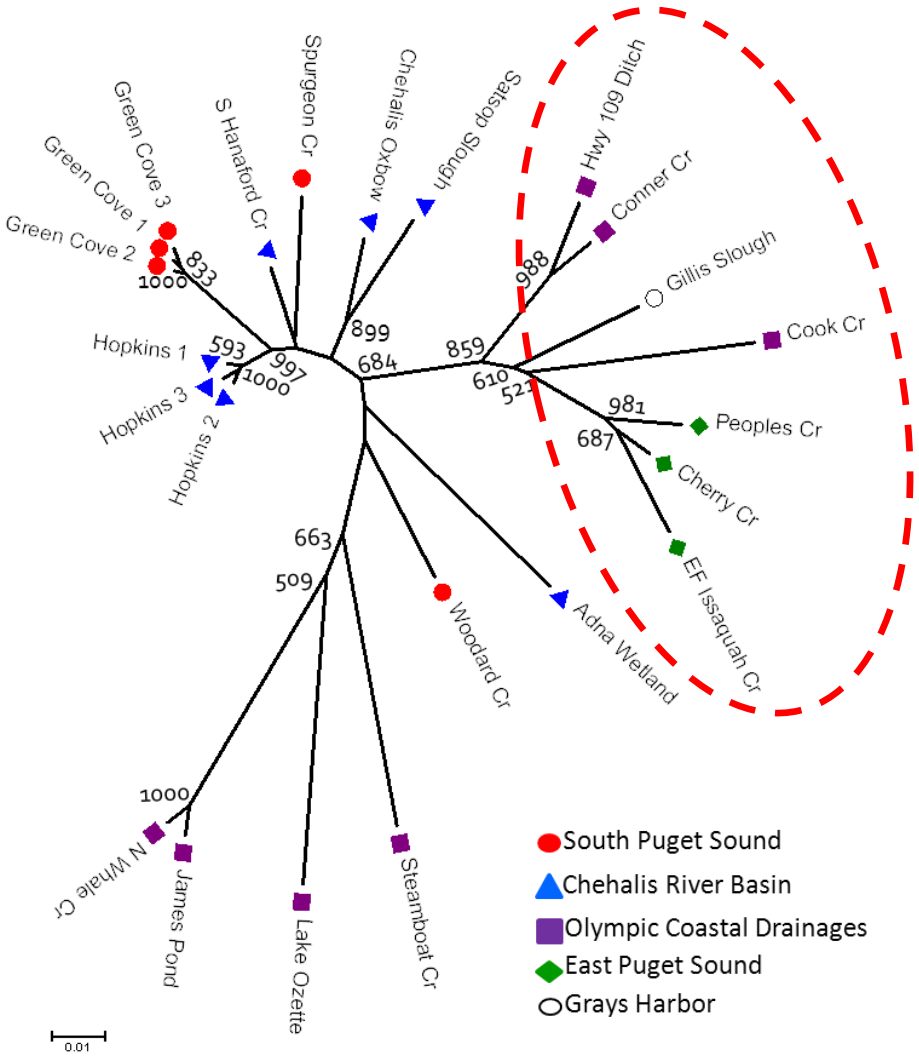


Trotter et al. (2000) NW Naturalist 81:59-63

Origins of E. Puget Sound Populations



Origins of E. Puget Sound Populations



F_{ST} COMPARISONS AMONG WATERSHEDS

- E. Puget Sound vs. Chehalis $F_{ST} = 0.268$
- E. Puget Sound vs. S. Sound $F_{ST} = 0.311$
- E. Puget Sound vs. S. Olympic Coast $F_{ST} = 0.205$
- E. Puget Sound vs. N. Olympic Coast $F_{ST} = 0.410$

F_{ST} vs. R_{ST} Comparisons

- When populations have recently diverged, genetic differences are mainly due to drift; F_{ST} similar to R_{ST}
- When populations are historically diverged, genetic differences are also due to stepwise mutations; $R_{ST} > F_{ST}$
- Estimated both F_{ST} and R_{ST} and used permutation tests to compare

Population Pairs where R_{ST} is NOT significantly greater than F_{ST}

- 30 of 253 total comparisons were not significant
- 11 of those 30 involved E. Puget Sound populations

Sample Site 1	Sample Site 2	F_{ST}	R_{ST}	*P*-value
Peoples Creek	Cherry Creek	0.061	0.159	0.106
Peoples Creek	Conner Creek	0.200	0.243	0.050
Peoples Creek	Cook Creek	0.301	0.245	0.145
Peoples Creek	EF Issaquah Creek	0.126	0.112	0.571
Peoples Creek	Gillis Slough	0.153	0.155	0.122
Cherry Creek	Cook Creek	0.252	0.238	0.134
Cherry Creek	EF Issaquah Creek	0.080	0.028	0.848
Cherry Creek	Gillis Slough	0.096	0.084	0.253
EF Issaquah Creek	Cook Creek	0.325	0.229	0.436
EF Issaquah Creek	Gillis Slough	0.198	0.113	0.619
EF Issaquah Creek	Hwy 109 Ditch	0.226	0.334	0.068

Origins of E. Puget Sound Populations

- E. Puget Sound populations are most genetically similar to S. Olympic Coast, not Chehalis Basin or S. Puget Sound
- Based on genetic data these populations appear to be transfers of coastal fish
- E. Puget Sound populations have diverged from all other populations and from each other
- Genetic data does not suggest a specific source population

Conclusions

- ✓ Levels of genetic diversity varied across the species range and appear adequate to avoid any short term risks
- ✓ Nearly all of the sampling locations constitute a separate population with limited gene flow among populations
- ✓ Populations can be organized into genetic groups with the greatest amount of variation observed among 3 or 6 different groups
- ✓ Populations from E. Puget Sound are most genetically similar to S. Olympic Coast populations and likely represent introductions

Photo Credit: Roger Tabor

Acknowledgements

- Funding for this study provided by the USFWS, Washington Fish and Wildlife Office
- Numerous individuals assisted with genetic sample collections
  - Molly Hallock (WDFW), Teal Waterstrat (USFWS), Kira Mazzi (USFWS), Dan Lantz (USFWS), Larry Gilbertson (Quinault Tribe), Pat Trotter (retired), Dan Spencer (USFWS), Hans Berg (King County), Pat Crain (NPS), John Trobaugh (WDNR)
- Genetic Identification Services helped develop microsatellite markers
- AFTC Conservation Genetics Lab staff provided insightful discussion